

CLAIMS

1. A method for analysis of a sample containing or suspected of containing at least one analyte, frequently a biologically active compound, said method comprising:

a) contacting said sample with a functionalized complex of a metal M, where M is a metal ion selected from the group consisting of a lanthanide having atomic number 57-71, an actinide having atomic number 89-103 and yttrium(III) having atomic number 39;

in a reaction medium under binding conditions, whereby said analyte when present either interacts with said complex to form a conjugate or competes for interaction with a binding material specific for interaction with said complex and with said analyte;

b) adding to said reaction medium a luminescence-enhancing amount of at least one energy transfer donor compound of yttrium or a 3-valent lanthanide element having atomic number 59-71, provided that the lanthanide element of said functionalized complex and a lanthanide element of said energy transfer donor compound are not identical,

c) subjecting said reaction medium to excitation energy in the range of 200-400 nm, whereby enhanced luminescence in the range of 500-950 nm is generated,

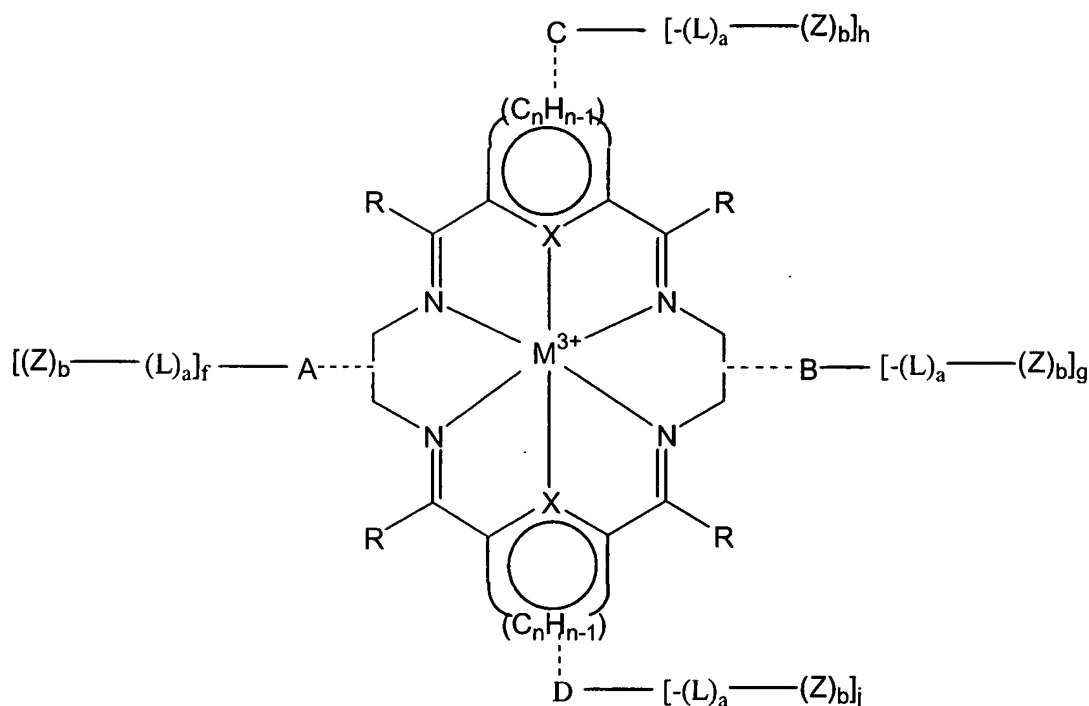
d) monitoring said luminescence of the reaction medium to measure in said sample at least one of the following:

(1) presence and/or concentration of said conjugate;

(2) presence and/or concentration of the product of the interaction of said complex with said binding material; and

(3) presence and/or concentration of the product of the interaction of the conjugate with the binding material.

2. The method of Claim 1 wherein said functionalized complex forms a compound having the formula



in which from one to two of A, B, C, and D are functionalized groups; L is a bridging/linking moiety between the functionalized macrocycle and a biologically active compound, Z is a residue of a biologically active compound linked to a functionalized group at A, B, C, or D directly or through L, a is zero or one, b is one, and each of f, g, h, and j is independently zero or one, provided that the sum of f, g, h, and j is either one or two.

3. The method of Claim 1 in which the analyte is a hapten having a molecular weight in the range of 125-2000 daltons.

4. The method of claim 3 in which the hapten is selected from the group consisting of

(a) Vitamins, vitamin precursors, and vitamin metabolites including retinol, vitamin K, cobalamin, biotin, folate;

(b) Hormones and related compounds including

(i) steroid hormones including estrogen, corticosterone, testosterone, ecdysone,

(ii) aminoacid derived hormones including thyroxine, epinephrine,

- (iii) prostaglandins,
- (iv) peptide hormones including oxytocin, somatostatin,
- (c) pharmaceuticals including aspirin, penicillin, hydrochlorothiazide,
- (d) Nucleic acid constituents including
 - (i) natural and synthetic nucleic acid bases including cytosine, thymine, adenine, guanine, uracil, derivatives of said bases including 5-bromouracil,
 - (ii) natural and synthetic nucleosides and deoxynucleosides including 2-deoxyadenosine, 2-deoxycytidine, 2-deoxythymidine, 2-deoxyguanosine, 5-bromo-2-deoxyuridine, adenosine, cytidine, uridine, guanosine, 5-bromouridine,
 - (iii) natural and synthetic nucleotides including the mono, di, and triphosphates of 2-deoxyadenosine, 2-deoxycytidine, 2-deoxythymidine, 2-deoxyguanosine, 5-bromo-2-deoxyuridine, adenosine, cytidine, uridine, guanosine, 5-bromouridine,
- (e) drugs of abuse including cocaine, tetrahydrocannabinol,
- (f) histological stains including fluorescein, DAPI
- (g) pesticides including digitoxin,
- (h) and miscellaneous haptens including diphenylhydantoin, quinidine, RDX.

5. The method of Claim 1 in which the analyte has a molecular weight greater than 2000 daltons.

6. The method of claim 5 in which the analyte is selected from the group consisting of polyaminoacids, polypeptides, proteins, polysaccharides, nucleic acids, glycosaminoglycans, glycoproteins, ribosomes and

- (a) proteins and their combinations including
 - (i) albumins, globulins, hemoglobin, staphylococcal protein A, alpha-feto-protein, retinol-binding protein, avidin, streptavidin, C-reactive protein, col-

lagen, keratin,

- (ii) immunoglobulins including IgG, IgM, IgA, IgE,
- (iii) Hormones including lymphokines, follicle stimulating hormone, and thyroid stimulating hormone,
- (iv) enzymes including trypsin, pepsin, reverse transcriptases
- (v) cell surface antigens on T- and B-lymphocytes, i.e. CD-4, CD-8, CD-20 proteins, and the leukocyte cell surface antigens, such as described in the presently employed CD nomenclature;
- (vi) blood group antigens including A, B and Rh,
- (vii) major histocompatibility antigens both of class 1 and class 2,
- (viii) hormone receptors including estrogen receptor, progesterone receptor, and glucocorticoid receptor,
- (ix) cell cycle associated proteins including protein kinases, cyclins, PCNA, p53,
- (x) antigens associated with cancer diagnosis and therapy including BRCA(s) carcinoembryonic antigen, HPV 16, HPV 18, MDR, c-neu; tumor suppressor proteins, p53 and retinoblastoma,
- (xi) apoptosis related markers including annexin V, bak, bcl-2, fas caspases, nuclear matrix protein, cytochrome c, nucleosome,
- (xii) toxins including cholera toxin, diphtheria toxin, and botulinum toxin, snake venom toxins, tetrodotoxin, saxitoxin,
- (xiii) lectins including concanavalin, wheat germ agglutinin, soy bean agglutinin,
- (b) polysialic acids including chitin;
- (c) polynucleotides including
 - (i) RNAs including segments of the HIV genome, human hemoglobin A

messenger RNA,

(ii) DNAs including chromosome specific sequences, centromeres, telomere specific sequences, single copy sequences from normal tissues, single copy sequences from tumors.

7. The method of claim 1 in which said luminescence is monitored with time-gated fluorescence instrumentation.

8. The method of claim 1 in which said luminescence is monitored with fluorescence instrumentation that is equipped with a continuous light source.

9. The method of claim 1 in which said luminescence is monitored with fluorescence instrumentation which measures multiple samples that are each automatically positioned in the luminescence detection zone.

10. The method of claim 1 in which said luminescence is monitored with fluorescence instrumentation which permits the imaging of the analyte.

11. The method of claim 10 in which said fluorescence instrumentation permits the measurement of the analyte at various points in the image.

12. The method of claim 11 in which said fluorescence instrumentation measures, records, processes, and/or displays the spatial distribution of one or more analytes.

13. The method of claim 12 in which said fluorescence instrumentation is a digital fluorescence microscope.

14. The method of claim 12 in which said fluorescence instrumentation is employed for comparative genomic hybridization.

15. The method of claim 12 in which said fluorescence instrumentation measures the analytes on a microarray.

16. The method of claim 1 in which the luminescence of an analyte in a nonaqueous environment is monitored and measured.

17. The method of claim 1 in which the analyte is monitored and measured in the dry

state.

18. A spectrophotometrically detectable luminescent composition comprising water, a micelle-producing amount of at least one surfactant, at least 1×10^{-10} moles/liter of at least one energy transfer acceptor lanthanide element functionalized complex having an emission spectrum peak in the range from 500 to 950 nanometers, and a luminescence-enhancing amount of at least one energy transfer donor compound of yttrium or a 3-valent lanthanide element having atomic number 59-71, provided that the lanthanide element of said functionalized complex and the lanthanide element of said energy transfer donor compound are not identical.

19. The composition of claim 18, wherein said energy acceptor lanthanide element functionalized complex is a macrocycle.

20. The composition of claim 19, wherein said macrocycle contains at least nine ring atoms of which at least three are donor atoms.

21. A composition according to claim 19, in which the lanthanide macrocycle has eighteen ring members.

22. A composition according to claim 18 which is a cloudy solution.

23. The composition resulting from the transfer of a composition of claim 18 to a non-aqueous environment.

24. The composition resulting from the transfer of a composition of claim 18 to a non-aqueous environment and removal of water.